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PKR and eIF2α: Integration of Kinase Dimerization, Activation, and Substrate Docking

The antiviral RNA-dependent protein kinase, PKR, binds to viral double-stranded RNA in the cell and halts protein synthesis by phosphorylating the α subunit of the translation initiation factor eIF2. In this issue of *Cell*, two complementary papers Dar et al. (2005) and Dey et al. (2005) address the interaction between PKR and eIF2α. The structures of eIF2α bound to PKR reveal that PKR forms a dimer, the interface of which is essential for kinase activation, and demonstrate how this protein substrate docks to its kinase. The structures, coupled with mutagenesis analysis, also demonstrate how phosphorylation of the activation loop can allosterically couple two distal regions, the dimerization and substrate recognition interfaces.

A common strategy in the cellular response to stress signals is to shut down protein synthesis. The eIF2α kinases are activated by various stresses to halt translation by phosphorylating, and thereby inhibiting, eIF2α, a protein involved in translation initiation. In humans, there are four eIF2α kinases—PKR, HRI, PERK, and GCN2—that are activated in response to viral double-stranded RNA (dsRNA), heme levels, misfolded proteins, and amino acid deprivation, respectively. In this issue of *Cell*, Dar et al. (2005) describe the structure of a complex between PKR and eIF2α. The structure is remarkable in several regards. It sets a new standard by representing the first kinase to be crystallized with a full-length protein substrate. It also shows how dimerization contributes to kinase activation and highlights the role of the αG-helix of PKR (described below) as a docking motif for eIF2α. The structural predictions are supported by mutational studies in Dey et al. (2005). The coupling of dimerization and substrate docking, mediated by phosphorylation of the activation loop, is revealed to be an ordered, highly dynamic, and extended allosteric process. The recent publication of a structure of GCN2 in the absence of eIF2α allows us to further appreciate the synergy between activation and substrate docking (Padyana et al., 2005).

To appreciate the importance of the PKR:eIF2α structure, one needs to summarize the general features of the protein kinase superfamily. Like all eukaryotic protein kinases, PKR has a smaller, more dynamic amino-terminal lobe (N-Lobe) and a larger, stable, mostly helical carboxyl-terminal lobe (C-Lobe). Two events are necessary to stabilize the active conformation. In the N-lobe, correct positioning of the αC-helix is essential, whereas in the C-lobe the activation loop typically must be phosphorylated. This phosphate then interacts with a conserved His-Arg-Asp (HRD) motif that precedes the catalytic loop at the active site. This interaction of the phosphate with the HRD arginine stabilizes the active site (Johnson et al., 1996) (Nolan et al., 2004). Three very stable helices, (αE, αF, and αH), form the core of the C-lobe, whereas the αG-helix, in contrast, is more solvent exposed (Yang et al., 2005).

Dar et al. (2005) describe the crystal structures of two complexes of PKR and eIF2α. Both form a symmetrical dimer, in one case related by crystallographic symmetry. The dimer interface primarily involves the N-lobe of PKR and the importance of this interface for dimerization and activation is confirmed in the accompanying mutagenesis study from Dey et al. (2005). These studies demonstrate how essential it is to determine the structure of more complete kinase-substrate complexes in order to understand the detailed mechanistic features of protein kinase activation and protein phosphorylation. As the kinase core is highly conserved, each kinase is activated and interacts with its substrates in new ways. In the case of eIF2α, this structure of PKR:eIF2α explains why a peptide containing the phosphorylation site is a poor substrate, unlike full-length eIF2α. EIF2α, and many other protein substrates, docks to kinases by additional "tethering" sites that lie peripheral to the active site. The structure of PKR and eIF2α suggests that substrate docking is a highly dynamic process where eIF2α docking to a distal site may actually contribute to the organization of the active site. Without the structure of the kinase-substrate complex, we could not appreciate the complexity and synergy between these two regions.

Additionally, the structure of eIF2α bound to PKR demonstrates the importance of the αG-helix of PKR as a substrate-docking motif, a function that is likely to be conserved in many protein kinases. The importance of the αG-helix for docking of proteins (see Figure 1) was demonstrated in three previous structures: the cdk2:KAP structure where cdk2 is the substrate for the KAP phosphatase (Song et al., 2001), the RIα inhibitory subunit bound to the catalytic subunit of PKA (Kim et al., 2005), and the autoregulatory domain bound to PAK1 (Lei et al., 2000). Interestingly, viruses also take advantage of this docking site. By competing for the eIF2α docking site on PKR, Dar et al. (2005) show how K3L, a vaccinia protein, likely could prevent the shutdown of protein synthesis in response to viral entry. In PKR, unlike the other kinases, the αG-helix assumes an atypical position that is tightly coupled to the kinase activation loop thus creating a new allosteric network that links substrate docking to dimerization (see Figure 2). Additionally, Arg499, conserved in all eIF2α kinases at the C terminus of the αG-helix, contributes to both positioning of this helix and coupling to the activation loop. An unresolved issue in this study is whether the αG-helix is always in an atypical position or whether this position is induced by eIF2α. In the GCN2 structure, with no bound eIF2α, the αG-helix is still in an atypical position indicating that this is likely to be an intrinsic feature of the eIF2α kinases (Padyana et al., 2005).

Another unusual feature revealed by the eIF2α complex is the region flanking the eIF2α phosphorylation

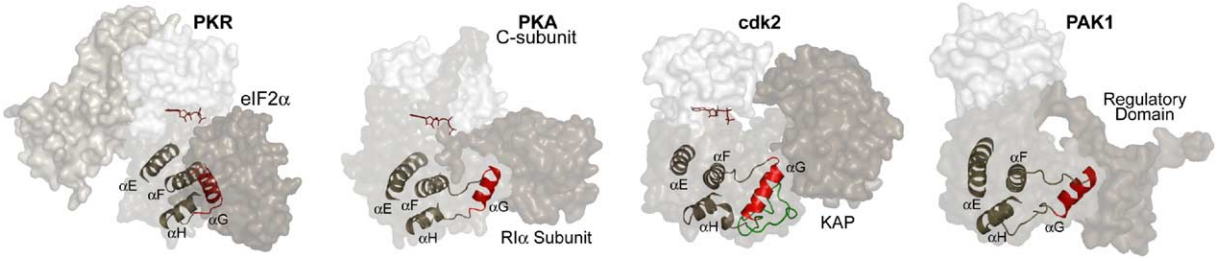

Figure 1. αG-Helix as a Docking Motif

The αG-helix (red) is involved in docking of cdk2 to the KAP phosphatase, RIα to the C subunit of PKA, and PAK1 to its regulatory domain. In PKR, we can appreciate clearly the docking function of the atypically oriented αG-helix to eIF2α and how this motif is integrated with activation. In cdk2, the kinase insert is in dark green. In this case, this insert is anchored to the αG-helix and can be thought of as extending the protein-docking surface. In PKA, the αE, αF, and αH-helices (dark gray) are stable and shielded from solvent, whereas the αG-helix is solvent exposed (Yang et al., 2005). The ATP nucleotide is shown in dark red.

site, Serine 51. Although this segment is poised in close proximity to the active site, it is, nevertheless, disordered in the crystal structure. This region, helical in free eIF2α, appears to have "melted" as a consequence of binding to PKR. Interestingly, when Ser51 is replaced by a tyrosine, it can still be phosphorylated. The structure, supported by mutagenesis analysis, explains this ambiguity by demonstrating that specificity is conveyed by a peripheral docking site. Once docked, the actual residue being phosphorylated can be rather promiscuous for PKR and perhaps for most dual-specific protein kinases. Furthermore, mutagenesis demonstrates that dimerization and autophosphorylation can be achieved even when the eIF2α docking site is nonfunctional indicating that substrate binding is not required for kinase activation, at least for a nonspecific peptide substrate.

The two requirements for activation, phosphorylation of the Thr446 in the activation loop and correct orientation of the αC-helix, are achieved for PKR by dimerization of the N-Lobe. As seen in Figure 2, by interacting with the HRD Arginine 413, the phosphate couples the activation loop to the active site. Typically, several other basic residues interact with the phosphate. In PKR, Lys304, and Arg307 in the αC-helix fill this role thereby stabilizing the helix in its active conformation. This allows a conserved glutamate in the αC-helix (Glu308) to interact with a conserved lysine (Lys296) in β strand three, an interaction that is important for catalytic activity. Although GCN2, bound to AMP-PNP, has the same

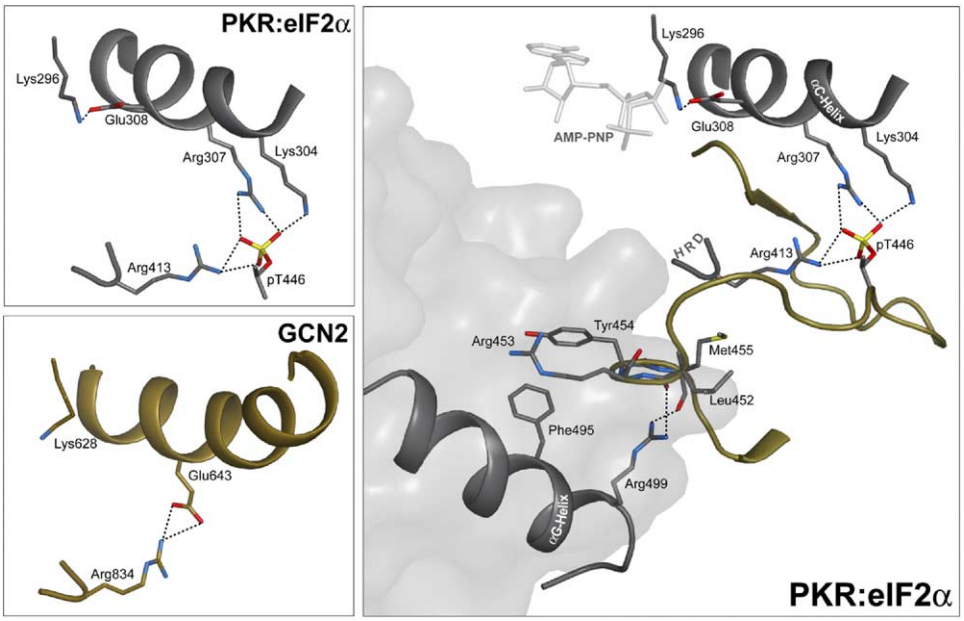

Figure 2. Activation and Coupling of Distal Sites by Phosphorylation of the Activation Loop

(Top left) The autophosphorylated active form of PKR shows the multivalent coordination of phosphorylated Thr446 (pT446) in the activation loop and the electrostatic pairing of Glu308 and Lys296. (Lower left) In GCN2, the αC-helix is twisted such that Glu643 in the αC-helix is interacting with the HRD Arg834 thereby contributing to stabilization of the inactive conformation. In spite of dimerization, GCN2 is not phosphorylated on Thr887. (Right) Depicted is the coupling of the phosphorylated activation loop in eIF2α to the dimerization site through the αC-helix, to the substrate docking site through the αG-helix, and to the active site through the HRD Arg413.

dimer interface as eIF2α, related by crystal packing, it is still not in an active conformation. It is not autophosphorylated and in the absence of phosphorylation of the activation loop, the conserved Glu643 residue of the αC-helix actually interacts with the HRD Arg834. GCN2 thus remains in an inactive conformation. Clearly dimerization is not sufficient to promote autophosphorylation or to snap the αC-helix into its active conformational state. GCN2, but not PERK and HRI, lacks both basic residues in the αC-helix, and this may explain why activation is not achieved so readily by simple dimerization. Although the precise mechanism remains to be elucidated, dimerization and phosphorylation of the activation loop appear to be closely linked for PKR, with dimerization being an essential first step.

Binding of a signal molecule to the N-terminal regulatory domain promotes dimerization and activation of each eIF2α kinase. PKR is activated by the binding of dsRNA to the N-terminal regulatory domain. In both the PKR and the GCN2 structures, the regulatory domain has been deleted. Therefore, how binding of dsRNA promotes PKR dimerization and activation cannot be resolved and is largely overlooked in these papers. In addition, the eIF2α kinases have a long β4-β5 linker. In both cases, this segment was truncated and what remains is disordered. Both regions could contribute directly to the activation mechanism; without the full-length structures it is premature to speculate on the ordered pathway for activation. Is the activation loop locked into an inhibited conformation in the absence of dsRNA in a way that involves the regulatory N-terminal domain or the β4-β5 linker? Does binding of dsRNA then release the inhibition? From the structure and mutagenesis studies it is clear that the allosteric mechanism for activation of the eIF2α kinases involves a tight and dynamic interaction between dimerization, autophosphorylation, and substrate docking. These studies reveal the intricacy of this integration and also demonstrate how phosphorylation of the activation loop can allosterically couple two remote sites.

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